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Nail morphology studies as assessments for onychomycosis treatment modalities

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Abstract

The purpose of this investigation was to study the morphology of the human nail treated with chemical penetration enhancers (CPE), bioadhesives and surface modifiers for assessment of topical treatment modalities for onychomycosis. CPEs, including dimethyl sulfoxide (DMSO) and urea were applied to human nail samples. Additional samples were treated with surface modifiers, tartaric acid (TTA) and phosphoric acid gel (PA). Other nail specimens were subjected to the bioadhesive polymers Carbopol® 971P and Klucel® MF. Atomic force microscopy (AFM), scanning electron microscopy (SEM) and polarized light microscopy (PLM) were utilized to visualize nail morphology and topographical changes of the human nail samples subjected to the various chemical agents. AFM, SEM and PLM micrographs revealed changes in topography to the dorsal layer when CPEs and surface modifiers were applied. Roughness scores as determined by NANOSCOPE™ IIIA software indicated a 2-fold increase when the dorsal nail layer was subjected to PA versus the control (147.8 vs. 85.0 nm, respectively). In contrast, when carbomer 971P was applied to the dorsal surface, roughness scores decreased significantly (44.6 vs. 85.0 nm, respectively). AFM, SEM and PLM studies of the human nail subjected to various chemical agents may be useful in the design and formulation of novel drug delivery systems for the topical treatment of onychomycosis. The AFM studies offer both a qualitative and quantitative assessment for nail treatment opportunities.

Keywords: Atomic force microscopy; Nail; Scanning electron microscopy; Light microscopy; Onychomycosis; Penetration enhancer; Drug delivery system

1. Introduction

Numerous adults suffer from chronic fungal infections of the fingernails or toenails (i.e. onychomycosis). It has been determined that approxi-

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mately 18% of the world population is afflicted with a microbial infection of the nail plate (Scher, 1996; Gupta and Scher, 1998). These fungal infections are especially troublesome to people with compromised peripheral circulation such as the elderly and diabetic patients. Others afflicted with these infections include those in the medical field, ranchers, farmers, military personnel, and users of acrylic nail products.

Most such infections are caused by obligate aerobic fungal species, 90–95% dermatophytic (*trichophyton*) and 5–10% yeast-like fungi (*Candida albicans*), which infect the nail plate itself (Niewerth and Korting, 1999; Piraccini and Tosti, 1999; Schlefman, 1999; Tom and Kane, 1999; Tosti et al., 1999; Debruyne and Coquerel, 2001). *Aspergillus versicolor* is also an important non-dermatophytic fungi infecting toenails (Debruyne and Coquerel, 2001). Although not usually imposing a significant health risk, increased therapies with antineoplastic agents and a continually growing population of immunocompromised individuals have shown an increased incidence of systemic morbidity from this 'topical' disease process. For example, onychomycosis and similar infections have become more common and more intractable in recent years particularly in immunosuppressed patients, such as those infected with Human Immunodeficiency Virus (HIV) or subjects suffering from Acquired Immunodeficiency Syndrome (AIDS) (Gupta and Scher, 1998; Scher, 1999).

Such nail infections frequently cause the nail to become discolored and/or deformed. Thus, there is a strong desire among infected individuals to treat the infection and return their nails to a normal appearance. Indeed, several studies have shown that infections of the nail cause serious emotional and psychological impact on the affected individual. Patients with onychomycosis have lower ratings for mental and physical health, self-esteem, social functioning, and for work-related activities than do their healthy, unafflicted counterparts (Whittam and Hay, 1997). The economic impact of nail infections is also very significant (Lubeck et al., 1993).

1.1. Treatment modalities—systemic

Historic treatment of these infections has had limited success; furthermore, physicians are reluctant to treat what has been generally perceived as merely a cosmetic disfiguration with a systemic medication. Negative aspects associated with oral systemic antifungal therapy for onychomycosis include their limited success rate, contraindications and drug interactions, toxicity, and the high cost of the medication. Furthermore, a general movement has begun in the medical and scientific communities away from the use of systemic antimicrobial therapy because indiscriminate and widespread use of broad-spectrum antibiotics has lead to an increase in the number of resistant strains of pathogenic microorganisms (Evans, 1998; Scher, 1999).

Unfortunately, many fungal nail infections have proven to be very resistant to any type of treatment. Systemic administration of anti-fungal drugs, such as the azoles (ketoconazole, fluconazole) and the allylamines (terbinafine, butenafine), is hindered by limited blood circulation in the nail bed and poor transport to the nail plate, requiring high dosage levels for long periods of time. Such high drug dosages can have adverse side effects, and it has been found that clearance of the infection is often only temporary. Systemic treatment must often be continued indefinitely, thereby also increasing the potential for antimicrobial resistance.

1.2. Treatment modalities—topical

Topical therapy for onychomycosis might be the treatment of choice, since it does not lead to adverse systemic effects or drug interactions. However, topical administration of anti-fungal drugs also suffers limitations. This treatment modality has not been effective, because antifungal drugs cannot readily penetrate the nail plate to reach the infection sites under the nail. The nail plate is a relatively thick structure that inhibits penetration of the drug being applied (Walters et al., 1983; Gniadecka et al., 1998). Moreover, the topical application of creams, solutions, lotions and gels is often dissipated in relatively short

periods of time. Although attempts have been made to incorporate topically active antifungal drugs into film-forming compositions (e.g. nail polishes or lacquers to improve drug persistence), such approaches have not proved entirely satisfactory. While removal of the nail (nail avulsion) can improve topical drug treatment, several disadvantages to this treatment modality exist which include poor patient acceptance and the ability to maintain a constant supply of the drug to the nail bed. Another recent means of treating onychomycosis include laser therapy on the affected nail, followed by topical administration of an antifungal agent (Karell, 1999). However, laser therapy, as yet, is not well developed nor widely practiced, in addition to being very expensive.

Although the nail is similar to the stratum corneum of the skin in that it is derived from epidermis, it is composed primarily of highly disulfide-linked keratin and is approximately 100-fold thicker than stratum corneum (Walters et al., 1983; Gniadecka et al., 1998). The nail contains, what is generally agreed to be, three layers, with the dorsal layer being the most electron dense and resistant to antimicrobial penetration. Thus, in order to deliver a sufficient amount of drug into the nail plate, the permeability of the nail plate to the drug needs to be enhanced.

For these and other reasons, it is desirable to provide an effective system and composition for topically treating nail fungal infections. Specifically, a need exists to deliver a potent antimycotic agent through the nail to attain appropriate concentrations so that the targeted fungal organism is eradicated. A delivery system to achieve this need must possess the necessary chemical, mechanical or physical properties to allow penetration of the antimycotic through the three layers of the nail such that therapeutic levels are attained. These investigations of the nail at the microscopic level, coupled with permeability and other conventional studies, may lead to the development of a drug delivery system that achieves the above-discussed criteria.

The atomic force microscope (AFM) is a type of scanning probe microscope that can be used to image micron to nanometer-sized topography. As Patel and co-workers outlined, AFM studies are used in biological structure studies since the investigated specimen does not have to be electron or ion-conductive (Patel et al., 2000). In addition, this instrument can record morphological information in environments that other techniques cannot achieve (Binning et al., 1986). In AFM, a sharp tip (usually made of hard and non-reactive material such as silicon or silicon nitride) is mounted on a cantilever. As the cantilever moves toward the sample surface, the interaction forces between the tip and the surface will cause the cantilever to deflect due to the sample–tip interaction force. Any movement of the cantilever is detected by changes in a laser beam reflecting off the cantilever onto a split photodiode (Fig. 1). A three-dimensional image can be constructed and displayed with the appropriate software. There are several AFM operating modes, with the tapping mode atomic force microscope (TMAFM) providing the most reliable morphology, especially for biological or polymer surfaces (Weisenhorn et al., 1993; Camesano et al., 2000; Pietrasanta et al., 2000). In ambient air conditions, TMAFM can measure forces smaller than 10^{-7} N (Attard et al., 1999) and achieve a lateral resolution of 1–2 nm (Weihs et al., 1991).

Nail morphology via polarized light microscopy (PLM) and SEM has been investigated for decades (Thorndike, 1968; Montagna and Parakkal, 1974; Mauro et al., 1975; Germann et al., 1980; Baden and Kibilus, 1984; Baden, 1987) however, the research was not aimed at assessing drug delivery opportunities. Indeed, AFM studies for the investigation of the human nail have not been reported for any purpose.

The objective of this study is to compare the morphology of the non-treated human nail with those treated with chemical penetration enhancers (CPEs), bioadhesives, and surface modifiers for the partial assessment of topical treatment modalities for onychomycosis. This study, in addition to nail permeability and other appropriate investigations, will facilitate a more strategic development track for drug delivery systems via hot-melt extrusion technology, which is a topic of ongoing

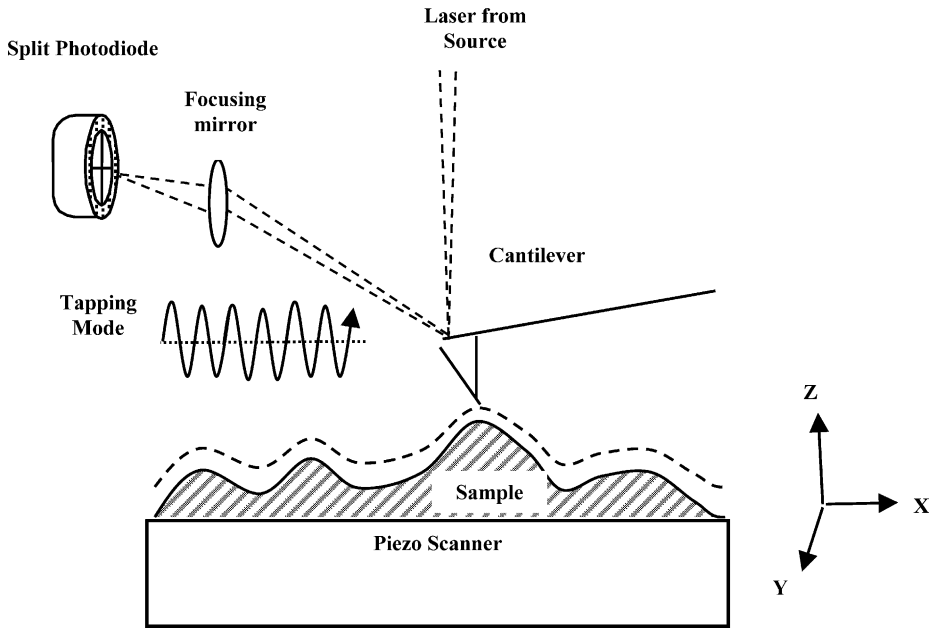

Fig. 1. Schematic drawing of an AFM.

and future work (Follonier et al., 1995; Repka et al., 1999; Zhang and McGinity, 1999; Repka and McGinity, 2000, 2001a,b).

2. Materials and methods

2.1. Materials

Tip nail pieces were obtained from the fingers of two human volunteers utilizing nail clippers. The samples were collected and immediately sealed in 4 ml polyethylene bags. The Klucel® MF (Hydroxypropylcellulose (HPC)) was kindly gifted by Aqualon, Division of Hercules, Inc., Wilmington DE. Carbomer 971P (Carbopol® 971P) was provided by BF Goodrich Specialty Chemicals (Cleveland, OH 44141). Dimethyl sulfoxide (DMSO), urea, tartaric acid (TTA) and other reagents were obtained from Spectrum Chemical. Phosphoric acid gel (PA) 10% was obtained as a gift from Dr A. Harvey Leslie, DMD (Oxford, MS).

2.2. Methods

2.2.1. Preparation of solutions and dispersions

The polymers used in this work were dissolved in nanopure water to give a 1.0% w/v solution. The dispersions were stored at 4 °C for at least 48 h to allow the polymer chains to fully hydrate prior to use. Other solutions were 20% w/v in either water or ethanol.

2.2.2. Procurement and treatment of nail samples

Tip nail pieces were obtained from the fingers of healthy volunteers using nail clippers. An aliquot of the solutions was placed in a test tube along with six nail samples and agitated for 30 min at 30 °C. Six dorsal nail surfaces were subjected to PA for 30 s using a dental sponge applicator. The TTA solution was placed on six samples in the same manner for 90 s. The excess polymer/chemicals were removed by washing with approximately 100 ml of distilled water for 3 min. The nail samples were carefully placed on a petri-dish and allowed to air dry for 30 min before visual examination. Six nail samples were used as controls. Both treated and untreated photomicro-

graphs illustrated in this study were from the same individual and the same finger.

2.2.3. Atomic force microscopy

Atomic Force Microscopy (AFM) studies were performed in ambient air (ca. 25 °C and 50% relative humidity) using a Multimode AFM (Digital Instruments Inc., Santa Barbara, CA). A J-type scanner was used in tapping mode with a maximum scan size of 125 μm and a scan rate of 1 Hz. The cantilever was 125 μm in length with a 4 μm silicon tip. The scanning frequency was close to or at the resonant frequency of approximately 348.5 kHz. Image analysis was performed by the NANOSCOPE IIIA V4.23 software. Images were flattened using third order, lease square fit before analyzing. Images were scanned utilizing a scanning force set point ratio between 0.7 and 0.9.

The root-mean square roughness was calculated as follows:

$$\mathrm{Rq} = \sqrt{\frac{\sum (Z_i - Z_{\mathrm{ave}})^2}{N}}$$

where Z_i is the height of a local point (average height of a pixel); Z_{ave} the average height in a given area (given amount of pixels); and N is the number of pixels in a given area.

2.2.4. Scanning electron microscopy

Samples were coated with gold–palladium for 60 s under an argon atmosphere using a Pelco Model 3 cold sputter module (TED Pella Inc., Tustin, CA) in a high vacuum evaporator equipped with an omni-rotary stage. The morphologies of the samples were investigated by using a Hitachi S-4500 SEM (Hitachi, Ltd., Ibaraki-Ken, Japan) at 15 kV. Two magnifications were selected-100 and 5000 ×.

2.2.5. Polarized light microscopy

A polarized light microscope (PLM) (Polam-L 213-TE, LOMO, PLC) was used to photograph the nail clippings. It was equipped with a digital camera to capture the nail specimen's morphological images.

2.3. Statistical analysis

Statistical analysis was determined utilizing one-way analysis of variance (ANOVA). A statistically significant difference was considered when $P < 0.05$.

3. Results and discussion

Human nail morphology has been studied by several investigators over the last three decades via PLM and scanning electron microscopy (SEM; Thorndike, 1968; Montagna and Parakkal, 1974; Mauro et al., 1975; Germann et al., 1980; Baden and Kibilus, 1984; Baden, 1987). However, no study to date has utilized AFM to investigate the nail's morphological structure for assessing drug

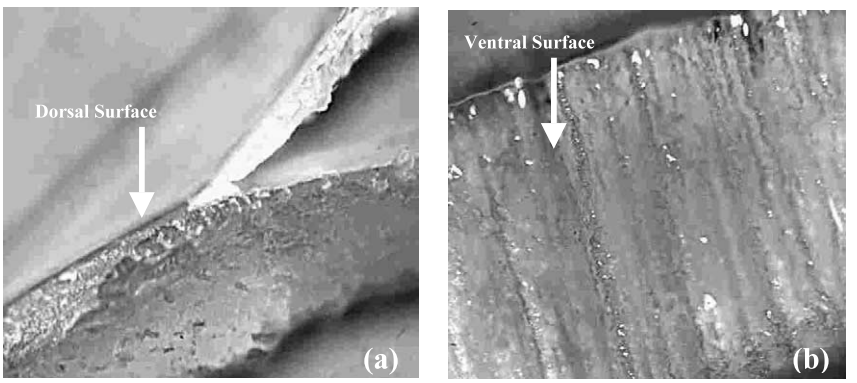

Fig. 2. Photomicrograph of a human nail illustrating (a) dorsal, intermediate and ventral layering, and (b) Eosin stained ventral surface.

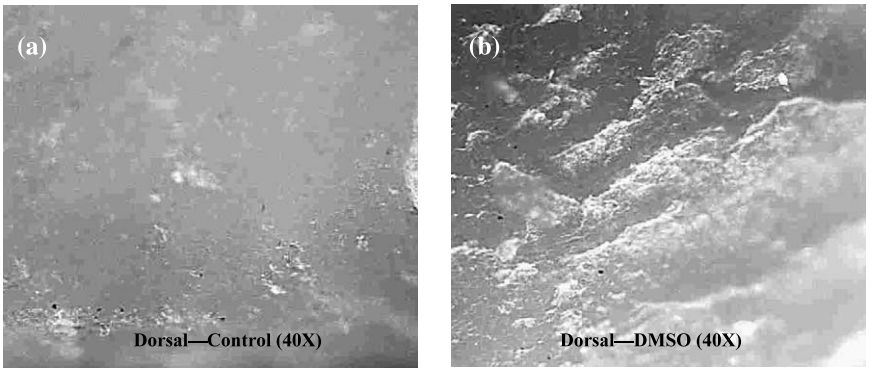

Fig. 3. Photomicrograph of a human nail illustrating (a) normal dorsal nail surface (40 ×); and (b) same dorsal surface treated with DMSO (40 ×).

delivery opportunities. Therefore, with drug delivery assessment in mind, this research uses three types of microscopy (i.e. PLM, SEM, and AFM), to compare the morphology of the human nail treated with CPE, bioadhesives and surface modifiers for the assessment of topical treatment modalities for onychomycosis.

PLM was employed to study the gross morphology of the human nail. Fig. 2a illustrates the layering of the electron dense dorsal layer of the nail. It is apparent that this highly disulfide-bond-linked layer is at least one of the primary barriers for topical drug penetration. Indeed, Kobayashi and co-workers concluded that the dorsal nail layer was the main barrier to drug penetration in the nail plate (Kobayashi et al., 1999). Fig. 2b demonstrates the ventral surface with its corrugated morphology for interdigitation with the epidermal rete within the nail bed. This grooved matrix system between the nail's ventral surface and the nail bed demonstrates that an effective topical treatment without nail avulsion is a much-desired therapy, which would reduce post-operative pain and minimize the disruption of the nail bed vasculature. Fig. 3 shows the dorsal surface of the nail before treatment (Fig. 3a) and after treatment with DMSO, a well-known skin penetration enhancer. Note the increase in corrugation in the DMSO treated surface. DMSO and other chemical enhancers have been reported to modify biological membranes by dissolution and/or disruption of lipid membrane packing order (Turunen et al., 1994; Kanikkannan et al., 2000; Barry, 2001; Takahashi and Rytting, 2001). Kobayashi et al. concluded that the ventral and dorsal layers in

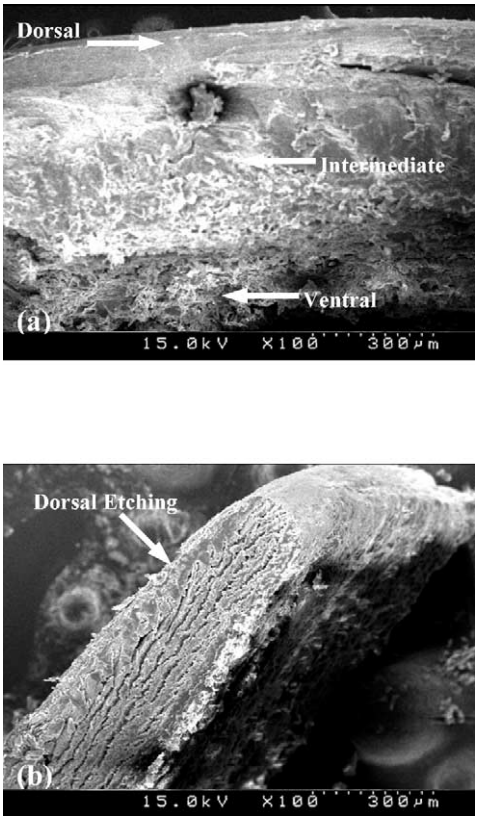

Fig. 4. Scanning electron micrographs of (a) the cross-section of a human nail plate (100 ×), and (b) the cross-section of a human nail plate exposed to TTA (100 ×).

the human nail plate contain some lipids (ventral > dorsal), whereas the intermediate layer, which comprises most of the nail, contains few lipids (Kobayashi et al., 1999). These investigators also determined that the nail plate functioned as a hydrophilic-gel membrane, confirming studies done previously by Walters et al. As has been reported by numerous investigators, it is quite apparent that the nail is comprised of a much smaller quantity of lipids than that of the epidermis (Walters et al., 1985; Baden, 1987; Kobayashi et al., 1999). Walters and co-workers concluded that solvents that promote diffusion through the epidermis do not have much promise as penetration enhancers through the nail plate (Walters et al., 1985). Thus DMSO and other skin penetration enhancers may not be the most effective way to enhance drug penetration through the nail via topical treatment.

Cross-sectional views via SEM are seen in Fig. 4. At 100 ×, the dorsal surface is intact in the non-treated nail sample (Fig. 4a). However, in Fig. 4b, upon treatment with the surface modifier, TTA, one notices the disruption, and in some cases, the complete removal of the dorsal surface, which was taken from the same nail sample. It may be argued that drug delivery through this treated nail would be enhanced due to the micrographs demonstrating these morphological changes. SEM is also utilized in Fig. 5 to illustrate the dorsal surface of the nail at the magnification of 5000 ×. Fig. 5a is the control or non-treated surface. Note the different morphological changes when the dorsal surface is subjected to urea (Fig. 5b), TTA (Fig. 5c) and PA (Fig. 5d). The PA treated dorsal nail indicates the most disruption of surface morphology. This two-dimensional SEM suggests a highly etched surface, interrupting the integrity of the most impenetrable layer of the nail (Kobayashi et al., 1999).

The AFM utilized in this study is one of several types that can obtain topographic information of surfaces with minimal sample preparation (Binning et al., 1986). As Patel et al. (2000) outlined, AFM studies are used in biological structure studies since the investigated specimen does not

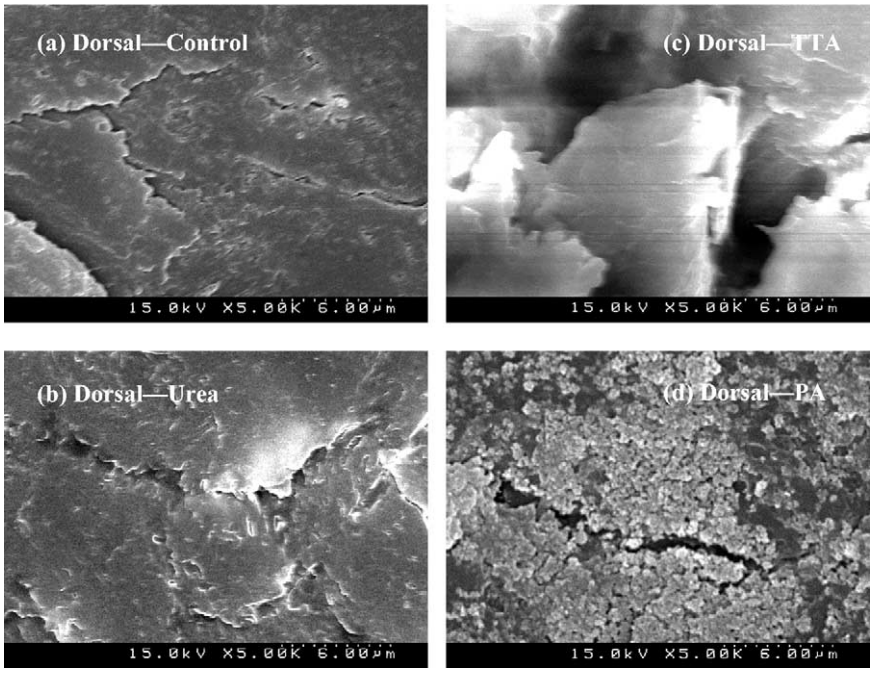

Fig. 5. Scanning electron micrographs of the human dorsal nail plate, (a) control (non-treated surface), and those exposed to (b) urea, (c) TTA and (d) PA.

have to be electron or ion-conductive. In addition, it can assess morphological information in environments that other techniques cannot achieve (Binning et al., 1986).

Fig. 6a and b are pseudo-three dimensional AFM representations of the dorsal and ventral nail surfaces, respectively. Note the broad valley of the ventral surface as compared with the slightly

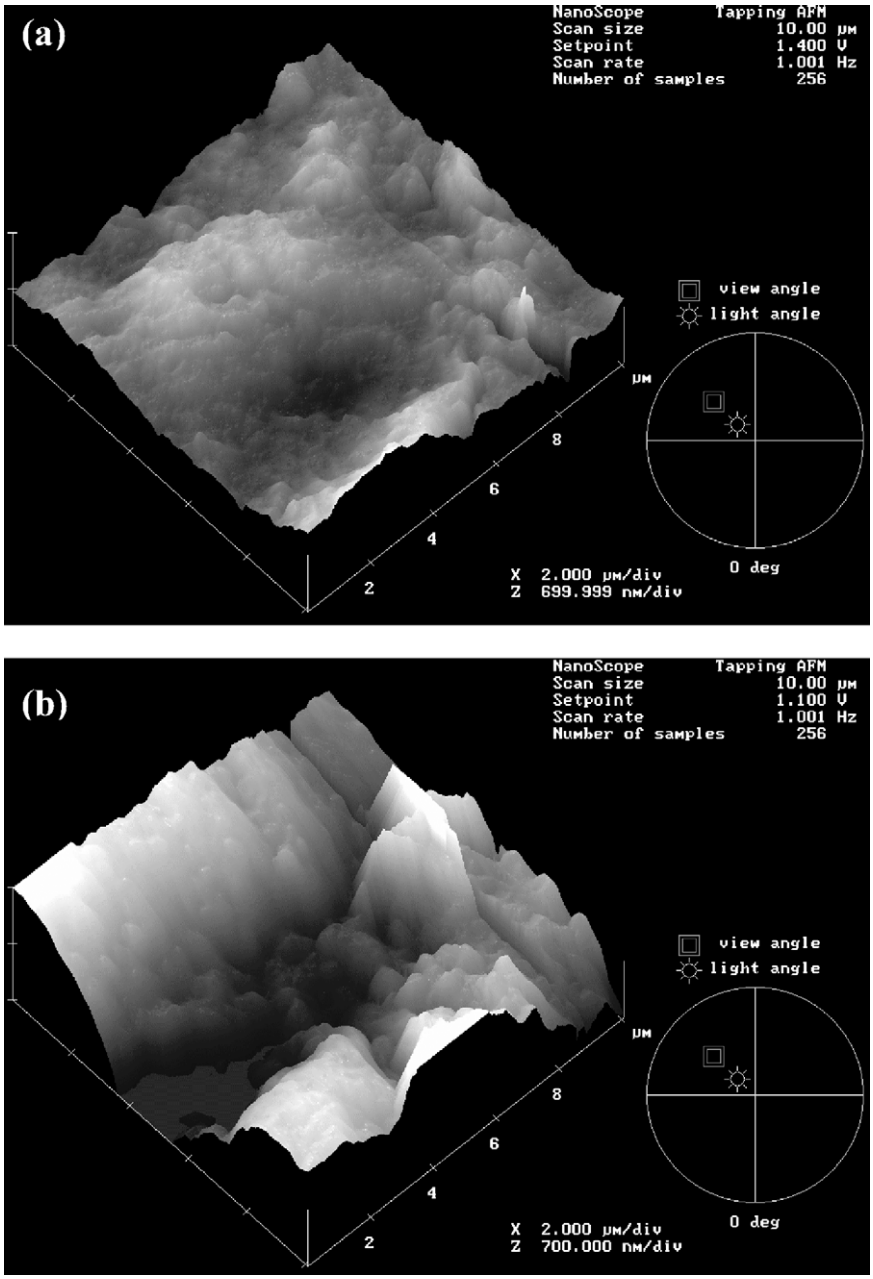

Fig. 6. Atomic force micrographs of the human nail representing (a) the normal dorsal surface, and (b) the normal ventral surface.

irregular dorsal surface. Fig. 7 illustrates the dorsal surface subjected to two polymer dispersions Carbopol® 971P (Fig. 7a) and Klucel® MF (Fig. 7b). The nail surface of the acrylic polymer in Fig. 7a is notably smoother than the granular topography of the surface containing the cellulosic polymer in Fig. 7b. However, both polymeric treated surfaces have a smaller peak to valley

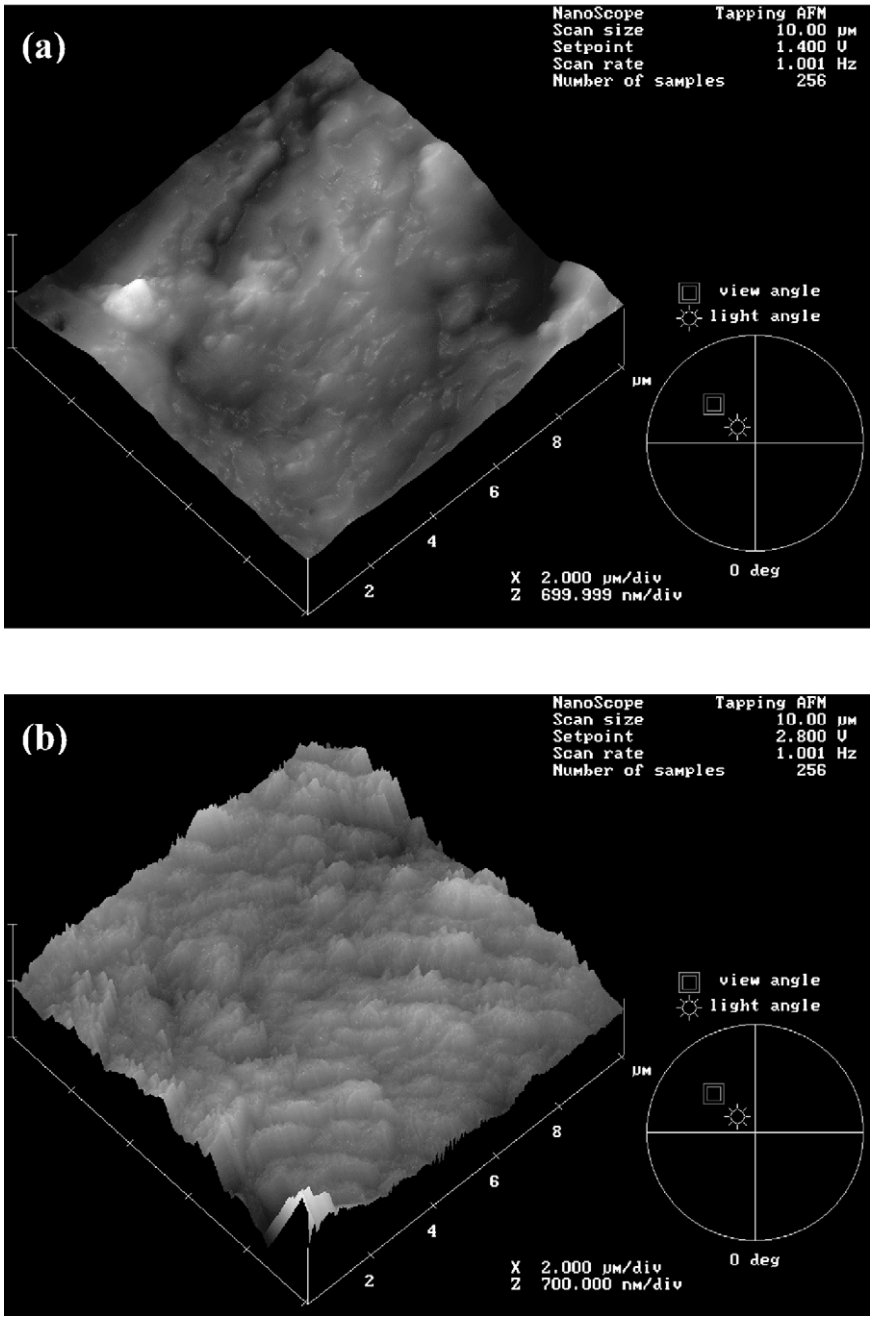

Fig. 7. Atomic force micrographs of the human dorsal nail plate; (a) treated with Carbopol® 971P; and (b) treated with Klucel® MF.

roughness than that of the non-treated dorsal surface (Fig. 6a).

The dorsal surface morphology changes quite dramatically, however, when subjected to the surface modifiers, TTA (Fig. 8a) and PA (Fig. 8b). Most notably, in Fig. 8b, one can observe the change in topography from the non-treated dorsal surface resulting in increased roughness and a consequent increase in surface area of the treated specimen. The following becomes apparent from

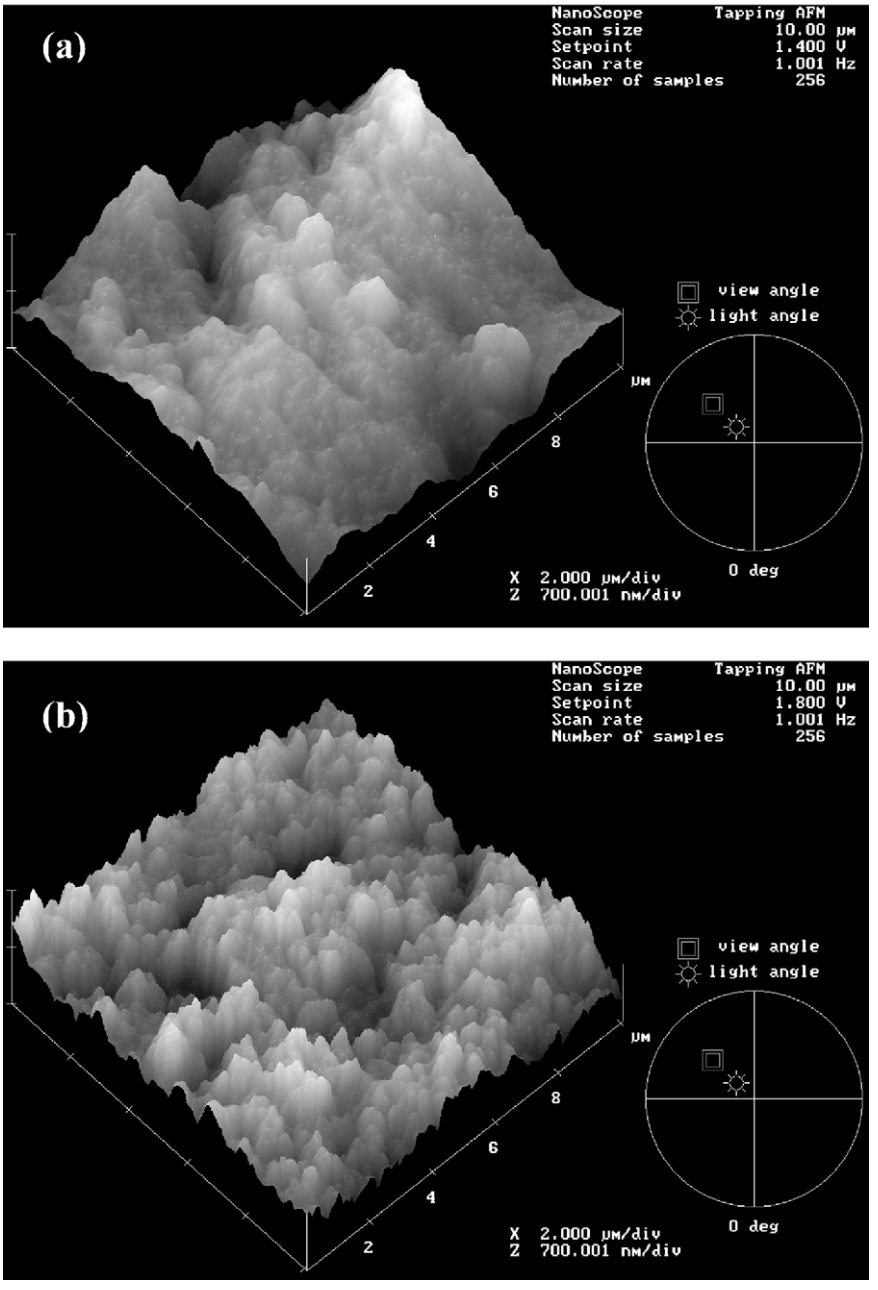

Fig. 8. Atomic force micrographs of the human dorsal nail plate; (a) treated with TTA, and (b) treated with PA.

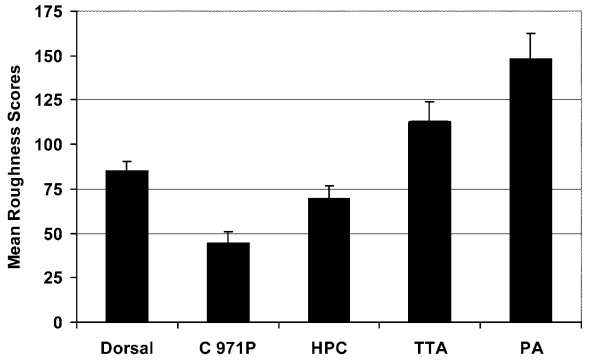

Fig. 9. Mean roughness scores of the dorsal surface of the human nail plate before and after chemical treatment (as determined by NANOSCOPE™ IIIA software).

the visualization of these two AFM micrographs: (1) the dorsal surface integrity is diminished such that an appropriate antifungal drug candidate would not have to diffuse through as thick of a layer as compared with that non-treated; (2) there is an increase in surface area such that drug diffusion can more readily occur; and (3) the increase in surface area provides a greater opportunity for polymer chains to inter-diffuse and bond with the nail plate-improving bioadhesion and retention of a drug delivery system (Lee et al., 2000). Thus, these morphological changes may benefit the topical treatment of nail infections, namely onychomycosis.

Quantitative topographic analysis via NANOSCOPE IIIA V4.23 software demonstrated significant differences in mean roughness scores of treated and non-treated dorsal nail surfaces. Fig. 9 is a graphic representation of these mean roughness scores of the non-treated, dorsal nail (control) and the treated samples. Both polymers effectively reduced the peak to valley roughness. The combined mean roughness scores for the two polymer-treated nails (44.6 and 69.7nm) were significantly lower than the control (85.6 nm) ($P < 0.05$). Bioadhesion studies are planned to determine the significance of this topographic observation with respect to drug delivery systems. Conversely, the mean roughness scores of the two surface modifier-treated nails were significantly higher (112.2 and 147.8 nm) than the control. This quantitative analysis supports the qualitative topographical images generated by AFM.

4. Conclusions

This investigation has demonstrated that AFM is a valuable technique for analyzing the human nail plate. This technique in combination with polarized light and SEM provides both qualitative and semi-quantitative information for the evaluation of nail morphology as it pertains to drug delivery. The impact of these morphological findings will be determined by permeation studies and bioadhesion testing in future work. However, the authors believe that assimilation of these types of images and their analysis, used in conjunction with conventional studies, can enhance the development of topical drug delivery systems for the treatment of onychomycosis and other disease states.

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References

Attard, P., Carambassis, A., Rutland, M.W., 1999. Dynamic surface force measurement II: friction and the AFM. Langmuir 15, 553–563.

Baden, H.P., 1987. The Nail, Diseases of the Hair and Nail. Year Book Medical Publishers.

Baden, H.P., Kibilus, J., 1984. A comparative study of the immunologic properties of hoof and nail fibrous proteins. J. Invest. Dermatol. 83, 327–331.

Barry, B.W., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 101–114.

Binning, G., Quate, C.F., Gerber, C., 1986. Atomic force microscope. Phys. Rev. Lett. 56, 930–933.

Camesano, T.A., Natan, M.J., Logan, B.E., 2000. Observation of changes in bacterial cell morphology using tapping mode atomic force microscopy. Langmuir 16, 4563–4572.

Debruyne, D., Coquerel, A., 2001. Pharmacokinetics of antifungal agents in onychomycosis. Clin. Pharmacokinet. 40, 441–472.

Evans, E.G., 1998. Causative pathogens in onychomycosis and the possibility of treatment resistance: a review. J. Am. Acad. Dermatol. 38, S32–56.

Follonier, N., Doelker, E., Cole, E.T., 1995. Various ways of modulating the release of diltiazem hydrochloride from hot-melt extruded sustained release pellets prepared using polymeric materials. J. Controll. Release 36, 243–250.

Germann, H., Barran, W., Plewig, G., 1980. Morphology of corneocytes from human nail plates. J. Invest. Dermatol. 74, 115–118.

Gniadecka, O.F., Nielsen, D.H., Christensen, H.C., Wulf, H.C., 1998. Structure of water, proteins, and lipids in intact human skin, hair and nail. J. Invest. Dermatol. 202, 393–398.

Gupta, A.K., Scher, R.K., 1998. Oral antifungal agents for onychomycosis. Lancet 351, 541–542.

Kanikkannan, N., Kandimalla, K., Lamba, S.S., Singh, M., 2000. Structure–activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr. Med. Chem. 7, 593–608.

Karell, M.L., 1999. Laser apparatus for making holes and etchings, USPTO, USA.

Kobayashi, Y., Miyamoto, M., Sugibayashi, K., Morimoto, Y., 1999. Drug permeation through the three layers of the human nail plate. J. Pharm. Pharmacol. 51, 271–278.

Lee, J.W., Park, J.H., Robinson, J.R., 2000. Bioadhesive-based dosage forms: the next generation. J. Pharm. Sci. 89, 850–866.

Lubeck, D.P., Patrick, D.L., McNulty, P., Fifer, S.K., Birnbaum, J., 1993. Quality of life of persons with onychomycosis. Qual. Life Res. 2, 341–348.

Mauro, J., Lumpkin, L.R., Dantzig, P.I., 1975. Scanning electron microscopy of psoriatic nail pits. New York State J. Med. 75, 339–342.

Montagna, W., Parakkal, P.F., 1974. Nails in the Structure and Function of Skin. Academic Press, New York.

Niewerth, M., Korting, H.C., 1999. Management of onychomycosis. Drugs 58, 283–296.

Patel, D., Smith, J., Smith, A., Grist, N., Barnett, P., Smart, J.D., 2000. An atomic force microscopy investigation of bioadhesive polymer adsorption onto human buccal cells. Int. J. Pharm. 200, 271–277.

Pietrasanta, L.I., Smith, B.L., MacLeod, M.C., 2000. A novel approach for analyzing the structure of DNA modified by benzo(a)pyrene diol epoxide at single-molecule resolution. Chem. Res. Toxicol. 13, 351–355.

Piraccini, B.M., Tosti, A., 1999. Drug induced nail disorders: incidence, management and prognosis. Dur. Saf. 21, 187–201.

Repka, M.A., McGinity, J.W., 2000. Physical–mechanical, moisture absorption and bioadhesive properties of hydroxypropylcellulose hot-melt extruded films. Biomaterials 21, 1509–1517.

Repka, M.A., McGinity, J.W., 2001a. Bioadhesive properties of hydroxypropylcellulose topical films produced by hot-melt extrusion. J. Control. Release 70, 341–351.

Repka, M.A., McGinity, J.W., 2001b. Influence of chlorpheniramine maleate on topical films produced by hot-melt extrusion. Pharm. Dev. Technol. 6, 295–302.

Repka, M.A., Gerding, T.G., Repka, S.L., McGinity, J.W., 1999. Influence of plasticizers and drugs on the physical–mechanical properties of hydroxypropylcellulose films prepared by hot-melt extrusion. Drug Dev. Ind. Pharm. 25, 625–633.

Scher, R.K., 1996. Onychomycosis: a significant medical disorder. J. Am. Acad. Dermatol. 35, S2–5.

Scher, R.K., 1999. Onychomycosis: therapeutic update. J. Am. Acad. Dermatol. 40, S21–26.

Schlefman, B.S., 1999. Onychomycosis: a compendium of facts and a clinical experience. J. Foot Ankle Surg. 38, 290–302.

Takahashi, K., Rytting, J.H., 2001. Novel approach to improve permeation of ondansetron across shed snake skin as a model membrane. J. Pharm. Pharmacol. 53, 789–794.

Thorndike, E.E., 1968. A microscopic study of the marmoset claw and nail. Am. J. Phys. Anthropol. 28, 247–261.

Tom, C.M., Kane, M.P., 1999. Management of toenail onychomycosis. Am. J. Health Syst. Pharm. 56, 865–871.

Tosti, A., Baran, R., Piraccini, B.M., 1999. Endonyx' Onychomycosis: a new modality of nail invasion by dermatophytes. Acta Derm. Venereol. 79, 52–53.

Turunen, T.M., Urtti, A., Paronen, P., Audus, K.L., Rytting, J.H., 1994. Effect of some penetration enhancers on epithelial membrane lipid domains: evidence from fluorescence spectroscopy studies. Pharm. Res. 11, 288–294.

Walters, K.A., Flynn, G.L., Marvel, J.R., 1983. Physicochemical characterization of the human nail: permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum. J. Pharm. Pharmacol. 35, 28–33.

Walters, K.A., Flynn, G.L., Marvel, J.R., 1985. Physicochemical characterization of the human nail: solvent effects on the permeation of homologous alcohols. J. Pharm. Pharmacol. 37, 771–775.

Weihs, T.P., Nawaz, Z., Jarvis, S.P., Pthica, J.B., 1991. Limits of imaging resolution for AFM of moleculars. Appl. Phys. Lett. 59, 3536.

Weisenhorn, A.L., Khorsandi, M., Kasas, S., Gotzos, V., Butt, H.-J., 1993. Deformation and height anomaly of soft surfaces studied with AFM. Nanotechnology 4, 106–113.

Whittam, L.R., Hay, R.J., 1997. The impact of onychomycosis on quality of life. Clin. Exp. Dermatol. 22, 87–89.

Zhang, F., McGinity, J.W., 1999. Properties of sustained-release tablets prepared by hot-melt extrusion. Pharm. Dev. Technol. 4, 241–250.